

## The Metabolic Cost of Click Production in Bottlenose Dolphins

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### LONG-TERM GOALS

Animals often increase the amplitude (the Lombard effect), duration, and/or repetition rate of their acoustic signals as a strategy to help reduce the probability of masking from environmental sounds (NRC 2003). Although accumulating evidence from recent research (Scheifele et al. 2005, Holt et al. 2009, Parks et al. 2010) illustrates that several marine mammal species readily modify the parameters of their acoustic signals to compensate for masking noise, potential energetic costs of such compensation behavior are unknown. To date, the only empirical data on the metabolic cost of sound production as well as the metabolic cost of increasing the amplitude of acoustic signals for any marine mammal species has been collected by the PIs during previously ONR-supported studies. The focus of the previous work was bottlenose dolphins producing whistles and other communicative sounds (Holt et al. 2011 a, b, Noren et al. 2011). There is currently no information on energy expenditure during click production in odontocetes, and studies have demonstrated that they also readily modify these sound types in an echolocation context to compensate for masking noise. Given that changes in vocal behavior in response to masking noise has been documented in several species, assessing the biological significance of these effects is paramount but also very difficult given the life histories of marine mammals. The Population Consequences of Acoustic Disturbance (PCAD) model has been proposed as a framework to address this challenging task (NRC 2005). Data on the energetic cost of the production of clicks from this study can be used to assess the biological significance of vocal compensation in response to sound exposure and populate transfer function 2 (transfer function between behavior change to life functions immediately affected) in the PCAD model.

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## OBJECTIVES

The objective of this study is to measure oxygen consumption in two captive bottlenose dolphins to determine the metabolic cost of click production. The metabolic cost of click production will then be compared to resting metabolic rates, the metabolic cost of whistles and other communicative sounds, and the metabolic costs of other activities, such as performing surface active behaviors (SABs) and/or swimming. This work requires two years to complete. Year 1 (training dolphins to perform the necessary behaviors and measuring metabolic rates during click production and resting trials) was initiated in 2012. For the second year of this study (ending in December 2013), we aim to increase the number of experimental trials in order to quantify the metabolic cost of click production in bottlenose dolphins. These measurements will quantify the potential metabolic cost of vocal compensation as an anti-masking strategy in response to anthropogenic sound exposure.

## APPROACH

The metabolic cost of click production is being measured in two captive male Atlantic bottlenose dolphins (*Tursiops truncatus*) maintained at Dr. Terrie Williams' Mammalian Physiology Laboratory at the University of California, Santa Cruz, Long Marine Laboratory. These individuals were trained by Traci Kendall (Program Manager/Research Training Supervisor) and Beau Richter (Head Trainer) to produce clicks on command while stationed under a metabolic hood to measure oxygen consumption. The sounds of free-ranging Atlantic bottlenose dolphins have been described as clicks, whistles, buzzes, quacks, and pops (Jacobs et al. 1993). The trained sounds of the captive dolphins of the current study are representative of those found in wild, free-ranging populations.

Experimental trials are conducted in the morning. The dolphins are fasted overnight before experimental trials to eliminate the potential for the metabolic cost of digestion to confound oxygen consumption measurements. Thus, food rewards are given after the experimental trial is complete and only one experimental trial is conducted per dolphin per day. Briefly, each experimental trial consists of one dolphin remaining at the water surface under the metabolic hood (details described in next paragraph) for one 10-minute period of rest at the water surface (to determine baseline metabolic rate), followed by two consecutive one-min bouts of click production (the two bouts are separated by 15 sec of silence) performed slightly below the water surface, and concluding with a recovery period at the water surface (at least 10 minutes, or until oxygen consumption values return to resting values). During all trials, the dolphins are acoustically monitored in real-time and their sounds are recorded for further analysis as described below. The total duration of the rest period, click production period, and recovery period are recorded for each experimental session. Respirations are also recorded during each of the three periods so that respiration rates can be calculated for the dolphins during rest, click production, and recovery. The dolphin's behavior during each trial is also video recorded to ensure that body movement is kept to a minimum during all trial periods (baseline rest, click period, recovery). For comparison, separate trials that measure oxygen consumption and respiration rates during rest in the absence of click production are also conducted (described in more detail in the work completed section). See figure 1 for a photograph taken during an experimental click trial.



**Figure 1.** Photograph taken during one experimental session showing the equipment set-up which includes the metabolic hood, the dolphin stationed under the metabolic hood, the acoustic recording equipment and operator, the dolphin trainer, and the assistant taking notes and recording respirations. During the trial the dolphin is acoustically monitored via a hydrophone attached by to the melon by suction cup (not shown) and all respirations are recorded during each of the three periods.

The method being used to determine metabolic rates from oxygen consumption values are similar to those used previously on bottlenose dolphins (Williams *et al.* 1993, Noren *et al.* 2011). For this study, the rate of oxygen consumption ( $\dot{V}o_2$ ) is being determined for quiescent dolphins stationed at the water surface and for the same dolphins producing clicks near the water surface. Air is drawn into the hood at a flow rate of  $300 \text{ L min}^{-1}$ . The flow rate is maintained such that the content of oxygen in the hood will remain above 20%. Water and  $CO_2$  from subsamples of excurrent air from the hood are absorbed using Drierite and Baralyme, respectively, prior to entering the oxygen analyzer. The percentage of oxygen in the sample line is monitored continuously (FMS field metabolic rate system, Sable Systems International) and recorded by a laptop computer every second during the experimental sessions.  $\dot{V}o_2$  for resting and vocalizing dolphins are calculated from the percentage oxygen data by respirometry software (Expedata data acquisition and analysis software, Sable Systems International). Dr. Dawn Noren is responsible for collecting and analyzing the respiration rate and oxygen consumption data.

All trials are acoustically monitored in real-time and also recorded using calibrated equipment to quantify the sound pressure level (dB re:  $1 \mu\text{Pa}$ ), duration (in sec), the repetition rate (clicks/min), the number of clicks, and the frequency and energy content of the clicks produced by each dolphin during the experiment. A contact hydrophone is placed on the dolphin's melon during trials to carefully quantify these click parameters. This method is being used because the dolphin is stationed at the air-

water interface under the hood and small changes in dolphin position can affect how much sound energy is transmitted under water. This will allow comparisons between trials and experimental conditions. The contact hydrophone consists of a Reson TC 4013 hydrophone that is molded into a small suction cup for contact. The contact hydrophone is then connected through a bandpass filter and amplified (Reson VP 2000). Then, the signal is sent through a DAQ device (IOTech Personal DAQ 3000) which digitizes the signal at a sampling rate of 500 kHz. The sound files are stored on a PC laptop for further analysis. Hydrophone placement is the same for each dolphin and during all periods (rest, click production, and recovery) of each experimental session. All sounds produced during trials are analyzed using Avisoft SASlab Pro (v5.1.17) and/or Matlab (R2011b or higher versions). Dr. Marla Holt is responsible for collecting and analyzing the acoustic data.

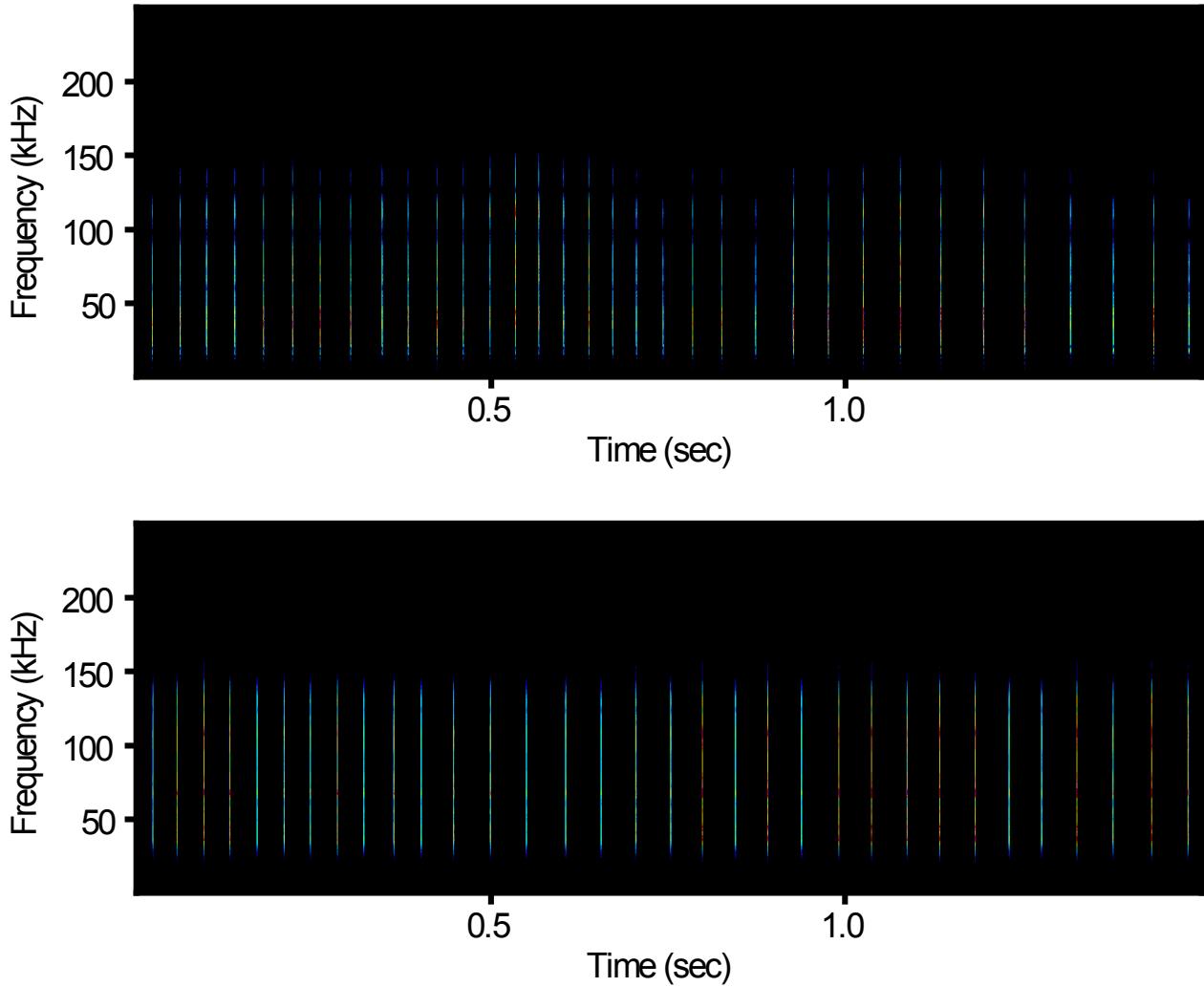
## WORK COMPLETED

In 2012, six trips to Dr. Terrie Williams' Mammalian Physiology Laboratory at the University of California, Santa Cruz, Long Marine Laboratory for data collection were completed. A brief (1 day) trip in April 2012 was conducted several weeks before the first schedule week of data collection to check on the status of the dolphins' click performance. During this trip it was realized that one of the dolphins (Primo) was producing very fast click trains which were more accurately described as burst pulses while the other dolphin's (Puka's) click trains were slower and more representative of echolocation behavior used for sensing objects during echolocation tasks. During the first full-week trip in May 2012, technical issues were tackled, and the final protocol for the experimental sessions was proposed. Due to issues with the two dolphins mastering the correct sound signal, additional training was required following this trip. A third, 2-day trip was conducted in early June to further evaluate the click performance of each dolphin. The fourth trip in late June was the first full-week trip when data collection officially began for both dolphins, although click performance was still variable for one dolphin (Primo) and some trials had to be thrown out. By early July, during the fifth full-week trip, both dolphins' click performance was stable for data collection. The sixth full-week trip was conducted in late-August and a seventh trip is scheduled to be completed in mid-October.

The focus of this first year has been to shape the performance of the dolphins' click signals and to slightly modify the protocol to ensure consistency of click performance across trials. For example, because the dolphins performed clicks are better when slightly submerged (which is the typical orientation for echolocating dolphins in the natural environment), the experimental trials were modified from the original proposal such that the dolphins now perform click bouts while slightly submerged and are only at the water surface during baseline, the 15 sec break between click bouts, and recovery. Because of the slight modification of the experimental protocol used when measuring the cost of whistles and communicative sound production, resting trials were also modified for the present study. Specifically, resting trials were run in a manner that mimicked the total trial duration and submergence pattern of click production trials. The total duration of resting trials are 22 min 15 sec and consist of three parts: 1) 10 min resting at the water surface, 2) one min resting slightly submerged, 15 sec resting at the water surface, one min resting slightly submerged, and 3) 10 min resting at the water surface. The purpose of conducting resting trials in this manner is to measure the metabolic cost of recovering from the two one min breath hold bouts in the absence of click production. By knowing the relative cost of breath hold bouts (determined from the resting trials), we will be able to isolate the cost of click production during the click production trials.

## RESULTS

A total of 44 experimental trials were conducted (22 per dolphin). Due to the difficulty of training these behaviors, only approximately 67% of these trials for Puka and 50% of these trials for Primo are currently deemed to be adequate for potential inclusion in future data analysis. Fig. 2 shows 1.5 sec examples of the clicks produced by each dolphin during a trial once click performance was stable and satisfactory for data analysis. Because dolphin performance during click trials improved with subsequent data collection trips, we will require additional data collection trips in 2013 to ensure an adequate sample size for statistical analysis and better assess which trials to include in the analysis.



*Figure 2. Spectrograms showing 1.5 second examples of clicks performed by Primo (top panel) and Puka (bottom panel) as recorded by a contact hydrophone placed on the melon of the dolphin. Both spectrograms show visual representations of clicks produced during oxygen consumption data collection with time from 0-1.5 seconds on the x-axis and frequency from 0-250 kHz on the y-axis. The colors denote relative level or amplitude differences with red indicating higher levels and blue indicating lower levels.*

## **IMPACT/APPLICATIONS**

Currently, there is no empirical data on the metabolic cost of click production in any delphinid species. Theoretical assessments of such costs need to factor in variables such as efficiency factors and the relationships between physiological processes and metabolic costs associated with behaviors given that they often do not simply scale according to linear relationships. However, such data needed for theoretical modeling on this topic are also lacking. Empirical data collected from this study will provide valuable information about the cost of click production in odontocetes and will be useful in assessing potential costs of modifying click production in response to anthropogenic sound exposure. Specifically, this study will provide important input data to populate transfer function 2 in the PCAD model which can then be used to assess the biological significance of such responses to anthropogenic sound exposure.

## **RELATED PROJECTS**

Dr. Terrie Williams' Marine Mammal Physiology Project involves other studies on the two dolphins used in this study. The goal of one related study is to assess the changing energetic demands in cetaceans, and in particular, determine the principle factors in regulating the variable metabolism of cetaceans over the seasons.

[http://www.mmpp.ucsc.edu/The\\_Marine\\_Mammal\\_Physiology\\_Project/Home.html](http://www.mmpp.ucsc.edu/The_Marine_Mammal_Physiology_Project/Home.html)

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## **PUBLICATIONS**

None yet.